PATENT SPECIFICATION

949,946

NO DRAWINGS

949,946

Date of Application and filing Complete Specification: Sept. 27, 1960.
No. 33190/60.
Two Applications made in United States of America (Nos. 845,103 and No. 845,104) on Oct. 8, 1959.
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COMPLETE SPECIFICATION

Improvements in or relating to Products for use in Healing

ERRATA

SPECIFICATION No. 949,946
Amendment No. 1

Page 1, line 50, *for* "epithelilization" *read* "epithelialization"
Page 5, line 93, *for* "effective" *read* "effecting"

THE PATENT OFFICE
22nd March 1966

for example, in areas of the human body where tissue has been lost or damaged as a result of injuries such as abrasions, cuts, burns, ulcerations and from surgical procedures and it is also concerned with the preparation of such materials.

Numerous approaches have heretofore been made and much study has been directed to problems involved in bringing about healing of areas where skin has been wholly or partially lost and where underlying tissues may have been damaged or removed as a result of burns, cuts, abrasions, ulcerations or from surgical intervention. The nature and character of the treatments currently used vary with techniques and preferences of individual surgeons and are dependent, also, on the nature, surface condition and extent and depth of tissue involvement.

A commonly employed procedure is that of viable skin grafting, in which the surgeon transplants shavings of living epidermal tissue from undamaged areas after suitable preparation. Such grafts have varied in size and thickness of the skin shavings used for grafting and in other details of procedure. Thiersch grafts, Ollier-Thiersch grafts, Wolfe grafts, pinch grafts, full-thickness and split-thickness grafts, and stamp grafts, are names associated with some of the various types of skin grafts and procedures which have been used. In preparing skin shavings for grafts, the surgeon [Pr

... the patient to provide autografts to cover the entire surface of the lesion. In such cases a portion of the lesion may receive an autograft and the remaining damaged area may receive grafts of living skin from another person, or of dead skin from a cadaver. Although such grafts, known as isografts, do not persist and do not become finally a part of the skin covering the healed lesion as autografts frequently do, they accomplish many desirable results such as preventing fluid loss, repressing the excessive development of granulation tissue, decreasing pain and stimulating growth of the stratum germinativum of the epidermis at borders of the lesion. Eventually isografts are rejected, that is, they slough off. It may be possible by then to cut additional skin grafts from the now-healed donor sites and so eventually to cover the lesion completely with viable autografts. Considerable effort has been directed to bring about the permanent survival of viable isografts but only slight progress has been achieved and no clinical applications of any of these studies have been widely accepted.

Although the isograft does not persist and become a part of the skin over the healed lesion it is of great value as a practical measure since, by use of isografts together with autografts, lesions with greater areas and degrees of tissue damage may be satisfactorily treated. However, the limited availability of human skin in relation to the needs

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I, IRENE NEUHAUSER, of 5759, South Kenwood Avenue, Chicago 37, State of Illinois, United States of America, a citizen of the United States of America, do hereby declare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention is directed to new and useful materials for stimulating and assisting healing processes in damaged mammalian tissues as, for example, in areas of the human body where tissue has been lost or damaged as a result of injuries such as abrasions, cuts, burns, ulcerations and from surgical procedures and it is also concerned with the preparation of such materials.

Numerous approaches have heretofore been made and much study has been directed to problems involved in bringing about healing of areas where skin has been wholly or partially lost and where underlying tissues may have been damaged or removed as a result of burns, cuts, abrasions, ulcerations or from surgical intervention. The nature and character of the treatments currently used vary with techniques and preferences of individual surgeons and are dependent, also, on the nature, surface condition and extent and depth of tissue involvement.

A commonly employed procedure is that of viable skin grafting, in which the surgeon transplants shavings of living epidermal tissue from undamaged areas after suitable preparation. Such grafts have varied in size and thickness of the skin shavings used for grafting and in other details of procedure. Thiersch grafts, Ollier-Thiersch grafts, Wolfe grafts, pinch grafts, full-thickness and split-thickness grafts, and stamp grafts, are names associated with some of the various types of skin grafts and procedures which have been used. In preparing skin shavings for grafts, the surgeon attempts to include portions of the stratum germinativum of the epidermis by cutting across the rete mucosum. When such grafts become attached to the surface tissues of the damaged area, they may become vascularized and form islands or centers of epithelilization on the surface of the lesion. Grafts for which the patient is the donor of the grafted skin are known as autografts.

It frequently happens that the size of the lesion is such that not enough undamaged skin areas remain on the patient to provide autografts to cover the entire surface of the lesion. In such cases a portion of the lesion may receive an autograft and the remaining damaged area may receive grafts of living skin from another person, or of dead skin from a cadaver. Although such grafts, known as isografts, do not persist and do not become finally a part of the skin covering the healed lesion as autografts frequently do, they accomplish many desirable results such as preventing fluid loss, repressing the excessive development of granulation tissue, decreasing pain and stimulating growth of the stratum germinativum of the epidermis at borders of the lesion. Eventually isografts are rejected, that is, they slough off. It may be possible by then to cut additional skin grafts from the now-healed donor sites and so eventually to cover the lesion completely with viable autografts. Considerable effort has been directed to bring about the permanent survival of viable isografts but only slight progress has been achieved and no clinical applications of any of these studies have been widely accepted.

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for isografts makes it important that other more readily available materials, capable of performing one or more of the functions of the isograft, be found and that practical procedures for their preparation and use should be developed.

An area of approach which has been extensively studied, extending back approximately eighty years, has involved the utilization of egg-shell membranes, the membrana testacea of eggs of domestic fowl. Numerous workers have utilized egg-shell membrane as a substitute for skin grafts but with varying degrees of success. In certain cases, where fresh egg-shell membrane was used in the repair of ruptured ear drums and as a graft to damaged areas from which skin had been entirely or partially lost, it was stated to have achieved reasonable success. In some cases, reported by other workers, fresh egg-shell membrane was found to be ineffective. Certain physiologists and surgeons concluded that satisfactory results could be obtained provided (1) that the egg-shell membrane pieces were applied with that surface towards the surface of the lesion which was previously in contact with the egg shell, and not the reverse, and (2) that the area was scarified until bleeding was produced prior to the application thereto of the egg-shell membrane. Unless these rules were carefully observed, according to certain of the workers in the art, the egg-shell membrane would not become "attached organically" to the denuded area to which it was applied. Another important criterion for the proper utilization of egg-shell membrane, according to early workers in the art, was to insure that, when applied with the shell-side to the lesion, it be in contact with the proliferating cells of the stratum germinativum of the skin at the periphery of the lesion. The said workers reported that the egg-shell membrane itself, and of its own initiative, takes no part in the formation of connective tissue but that the egg-shell membrane substance is transformed into connective tissue gradually, there being an interpenetration of the proliferating tissue into the structure of the membrane, followed by the establishment of circulation to care for the newly formed connective tissue structures.

Other workers have insisted that egg-shell membrane be applied with the albumen side toward the lesion, an exact reversal of the opinion of the above mentioned workers. The basis for this was the belief that the inner side of the inner shell membrane contained nucleated cells which served as foci for the healing processes. It is now known that such nucleated cells do not exist in egg-shell membranes. Still other workers employed the shell membranes from boiled eggs but disregarded the question of which side was placed in contact with the lesion.

Despite repeated investigation of the possible use of egg-shell membrane as a substitute for skin in grafting, the practice has not come into any appreciable or significant use. The difficulties of separating egg-shell membrane from eggs, the difficult problems of handling moist membrane pieces and the indifferent success which has attended the use of egg-shell membrane, as well as other factors, have all combined to make such approaches of dubious practical value.

In accordance with the present invention, highly important and significant improvements have been made which render the use of egg-shell membrane of great practical importance. It has been found, among other things, in accordance with the present invention, that egg-shell membrane can be treated in such a manner to convert it into such forms as to make its use highly practicable and of great value in meeting problems encountered in connection with successful clinical management of skin-denuded areas, deep tissue wounds and lesions which are refractory to the usual treatment procedures. These have heretofore frequently required the utilization of autografts and to a lesser extent, and with less success, the use of isografts. The present invention makes possible the production of novel egg-shell membrane products, which are highly effective for use in place of human skin in skin grafting operations, which are easily manipulated in use and which can be made by simple practical procedures. Moreover, in the various forms in which the novel egg-shell membrane products are produced, in accordance with the present invention, depending on the type and condition of the lesion to which they may be applied, they frequently display additional desirable properties such as a strong hemostatic action which tends to arrest bleeding, and a soothing, pain-relieving action, a type of action which some workers have reported to be a conspicuous property of isografts of human skin. Moreover, in the various forms in which the novel egg-shell membrane products are produced, in accordance with the present invention, they are readily and effectively storable so that they can be stockpiled in any desired amounts to meet emergency clinical situations which might be created due to holocausts, such as extended fires, wars and the like. These products constitute an effective treatment procedure providing a number of desirable characteristics and properties only some of which have heretofore been obtained by grafts of human skin.

The novel egg-shell membrane products made in accordance with the present invention play exceedingly important roles in tissue healing and grafting procedures. They serve, in fact, as effective biological dressings. In addition, by reason of their stimulating the regeneration of epithelial tissues and by providing topically certain factors which are

essential aids in the natural processes of tissue repair, they appear actively to promote the regeneration of tissues by stimulation and possibly by providing essential components for synthesis of the protein of the regenerated tissues.

The present invention involves the comminuting of egg-shell membrane to form a product having a greater surface area than that of the same weight of egg-shell membrane; such product may consist of separated fibrous and/or essentially non-fibrous particles prepared or derived from egg-shell membrane. Various effective procedures by which such may be accomplished are described in detail below.

According to the present invention, egg-shell membrane is reduced to exceedingly small particles preferably by disintegrative mechanical action. This is conveniently and very advantageously done in a high-speed homogenizing apparatus; excellent results are obtained with a homogenizer, the cutting blades of which can be caused to rotate up to a speed of approximately 45,000 rpm. Other types of equipment can, however, be used. The usual blenders employed in the home for preparing dispersions, disintegration and emulsification of food products have been found to give satisfactory results, especially if the rotating blades are first sharpened. Any other type of equipment may be employed provided it serves to convert the egg-shell membrane into finely divided form giving particles which may have a fibrous form or an essentially non-fibrous form depending upon the type of equipment employed and the details of procedure used.

Thus a product of a mainly non-fibrous form may be obtained by ball-milling the dried egg-shell membranes. Particles having mainly non-fibrous form may also be obtained by subjecting the dried egg-shell membranes to the action of a high speed homogenizer while the membrane pieces are suspended in air or in other suitable gases, or in a liquid which does not soften, or does not appreciably soften, the dried membrane as a result of imbibition of the liquid by the dried egg-shell membrane. Examples of such liquids are water-free methyl-ethyl ketone (2-butanone), 1:4-dioxane, benzthiazol, pyridine and many others.

Alternatively, particles having a mainly fibrous form are produced if the liquids used in the homogenizer are those which are readily imbibed by the dried egg-shell membranes and thereby cause the dried membrane to lose its crispness, to become flexible, swollen, and softened as in the intact egg. Examples of such liquids are water, aqueous solutions, methyl alcohol, glacial acetic acid, molten phenol crystals, formamide, propionic acid, lactic acid, chlorophenols and cresols. Numerous other non-aqueous liquids which soften and swell dried egg-shell membrane can be used, those above mentioned being simply illustrative.

Generally speaking, the liquids which soften and swell the dried egg-shell membranes and thereby condition the same for conversion by cutting, tearing or allied disintegrative action, as in a homogenizer, into a product comprising mainly separated egg-shell membrane fiber particles, are water-soluble or water-miscible and are of highly polar character, that is, the electron density is not uniformly distributed within the molecule. With some exceptions, of which glacial acetic acid is a particular example, these highly polar liquids have a high dielectric constant and are imbibed by the dried egg-shell membrane; and the extent to which such liquid is imbibed appears to be a measure of the extent of formation of separated egg-shell membrane fiber particles (since the keratin groups thereof are also of a polar character and are attracted to the liquid) when homogenization or disintegration in the homogenizer is effected. Some liquids which are very poorly imbibed by dry egg-shell membranes are appreciably imbibed when a relatively small amount of water is present. It is to be noted that various of the non-aqueous materials can be used in the form of aqueous solutions, mixtures or suspensions. Another characteristic of many of the non-aqueous liquids which are effective softeners for dried egg-shell membrane is that they have excellent hydrogen bonding tendencies. The softening and swelling of dried egg-shell membrane can also be effected by subjection of the latter to vapours of certain of the softening and swelling agents. For instance, in the simplest case, dried egg-shell membrane can be contacted with steam. It is preferred, however, to utilize liquid media, and especially aqueous media, in the distintegration of egg-shell membranes into fibrous particles.

The fibers of the product which results from disintegration in a softening liquid such as water have a diameter which is fairly constant and is generally between 3 to 7 microns, usually of the order of 4 microns when measured in the moist condition.

After homogenization or disintegration of the egg-shell membrane, the finely divided particles, both those with the essentially non-fibrous form and those having essentially or mainly the form of fibers, can be readily separated from the suspending liquid employed. This step is accomplished by filtration, by centrifuging, by evaporation of the suspending liquid or by other well-known procedures for separating solids from liquids. The adhering residue of suspending liquid, if any, is then removed from the egg-shell membrane particles by washing the particles with suitable solvents and these latter are removed by filtration, centrifugation, evaporation or other convenient means. Care must be taken not to

squeeze or compress the disintegrated or homogenized membrane mass as this tends to cause the formation of hard aggregates on drying. The resulting dry solid material consists of aggregates of small particles of disintegrated egg-shell membrane. The particles may be mainly non-fibrous in form or they may consist mainly of fibers, depending on the procedure used in their preparation. These dry aggregates of particles are easily disaggregated into separated particles by acting on the above described aggregates in the homogenizer in the absence of any suspending liquid, the aggregate pieces being disaggregated in air, or in other suitable gases, by high speed action of the homogenizer. The dry, fluffy mass of egg-shell membrane fibers, or the dry mass of separated air-dispersed or gas-dispersed mainly non-fibrous particles, depending on the procedure used to disintegrate the membrane, is now removed from the homogenizer. Hereinafter, for simplicity, the two types of products described above will be referred to as the "fluffy fiber pulp form," for the former, and simply as the "powder form" when referring to the latter. The disaggregation of the dry aggregates of either form in the homogenizer or other suitable high speed disintegrator requires but a short period of action at high speeds. Prolonged subjection of the products to treatment in the disaggregating apparatus at high speeds may build up elevated temperatures in the product which could damage it.

Pursuant to one aspect of the present invention, the fluffy fiber pulp form and the powder form of the disaggregated disintegrated egg-shell membrane substance may each be used, in their respective forms, separately or mixed together, for the therapeutic purposes previously described.

Pursuant to another aspect of the invention, the above described fluffy fiber pulp form, or the powder form of the disintegrated disaggregated egg-shell membrane, or a mixture of both forms, may be applied as a coating to cotton gauze, to paper, to woven and to non-woven fabrics, to sheets of perforated and imperforate synthetic plastics materials, or to other desirable base materials, on one or both sides, and the egg-shell membrane products may be made to adhere, if desired, to the surface of the base materials by means of suitable adhesive compositions, such as compositions containing pectin, gelatin, starch and/or starch derivatives, vegetable gums or soluble synthetic resinous polymeric substances and like innocuous adhesive materials.

In another important embodiment of the invention, a felt or felted sheet is formed in which the separated egg-shell membrane fibers of the fibrous form of disintegrated egg-shell membrane, mat together, or interlace, or become felted when these egg-shell membrane fibers, from boiled or unboiled egg-shell membranes, are laid down by procedures akin to those used in laying down felts of paper pulp, or sheets of paper in paper-making practice. To accomplish this aspect of the present invention it is desirable that the separated egg-shell membrane fibers contained in the disintegrated egg-shell membrane material be of sufficient average length to form a good felt or felted-fiber fabric or felted sheet from the suspension of egg-shell membrane fiber particles contained in the disintegrated egg-shell membrane suspension. In producing the aqueous suspension of egg-shell membrane fiber particles wherein a homogenizer is employed, it should be noted that if the separated fibers are subjected too long to the action of the cutting blades of the homogenizer the fibers may become progressively shorter and there will be an increased proportion of exceedingly fine particles. It is distinctly preferred that the disintegrating, homogenizing or pulping step be carried out to reduce the egg-shell membrane material mainly to a suspension of fibers or fiber pulp in which the fibers of the egg-shell membrane material are predominantly in the free form. Excellent results are obtained where the individual fiber particles are mainly of a length of 20 to 600 microns with an average within the range 60 to 300 microns, and of an average thickness or diameter of the order of 4 to 7 microns when measured in the moist condition.

The egg-shell membranes which constitute the starting material used in carrying out the invention can be separated from egg shells in any convenient manner. Thus, for example, this may be accomplished in a purely mechanical manner as, for instance, by rolling and pulling the membranes away from the washed egg-shells after removal of the yolk and albumen of fresh or uncooked eggs. In the case of cooked egg-shell membranes, these, too, may be removed by pulling them away from washed egg-shells which have been placed, for example, in boiling water to effect such cooking. In either case it is desirable to effect the separation of the egg-shell membranes from the egg-shells by grasping an edge of the membranes and rolling or pulling them away from the calcareous part of the egg shells.

Instead of employing purely mechanical means for effecting separation of the membranes from the egg shells, a combination of chemical and mechanical means can be utilized. One convenient procedure is to agitate, in a churn or the like, water-washed, very coarsely chopped or shredded egg shells containing the adhering membranes with several times their weight of a dilute acid, for instance, a 0.25 to 4% solution in water of mineral acids such as hydrogen chloride, or a 2 to 15% solution in water of organic acids such as formic acid, or acetic acid or lactic acid or propionic acid, for a period of time,

generally one to several hours, agitating until the membranes are loosened and fall away from the egg shell particles. The membranes may then be separated from the egg shell pieces by counter-current washing with water or aqueous solutions and by decantation steps. In case a small proportion of shell particles still remains attached to some membrane pieces, the membrane pieces are collected, washed with water by counter-current procedures and drained, then agitated for a period of one to four hours with a small amount of the dilute acid solution, followed by separation of the acid solution and counter-current washing of the membranes with decantation from bits of sedimented shell, using several changes of water.

The separation of the membrane from the shell may be effected with carbonic acid, that is, with an aqueous solution of carbon dioxide under pressure, though the process is time consuming. The time needed in this process may be decreased by agitating the shell pieces and by including with the shell material a cation exchange resin, for example, a cross-linked polystyrene polysulfonic acid, in the free acidic or hydrogen form, while the shell pieces are mixed with a solution of carbon dioxide in water, under compressed carbon dioxide. In these cases the separated membranes are finally washed with water or aqueous solutions using counter-current washing and decantation steps and the undissolved shell pieces and the cation exchange resin particles are separated with suitable sieves. The resin can then be regenerated to the free acid or hydrogen form by mineral acids, preferably hydrochloric acid, and is then ready for re-use.

In separating the membrane from the calcareous portion of the shell by means of dilute acids, separation is practically complete long before complete solution of the mineral part of the shell has taken place. It has been noted that the amount of mineral matter dissolved during the period necessary for membrane separation is much higher in the case of boiled egg shells than with uncooked egg shells, although the time of acid action is essentially the same. Evidently, boiling has created conditions in the shell which make more rapid the dissolving of the mineral matter by dilute acids.

The separated spent organic acid solutions may be regenerated for re-use conveniently by passing them through a column of appropriate cation exchange resin in acidic or hydrogen form and the cation exchange resins themselves may be regenerated to their acid forms with mineral acid solutions, such as dilute hydrochloric acid. Previous removal of the major portion of the dissolved calcium from the spent acid by the addition of sulfuric acid in amounts just insufficient to combine with all the calcium present and subsequent separation of the precipitated calcium sulfate by decantation and filtration, makes further regeneration of the dilute acid filtrate by treatment with cation exchange resins in hydrogen form a more efficient and economical procedure.

Another suitable way of effecting separation of the membranes from the egg shells is illustrated by the following procedure. Approximately 37 grams of water-washed egg shells containing adhering membranes are chopped and placed in an acid resistant (for example, enamelled) steel pressure vessel together with 1200 ml of 6% w/v acetic acid. The air in the vessel is displaced by carbon dioxide gas and then a pressure of 450 to 500 psi of carbon dioxide gas is maintained in said vessel for one hour. The carbon dioxide is then removed and suction by vacuum means is applied to the vessel. The suction is cut off, applied again, and again cut off. The vessel is then opened and the contents placed in a jar and agitated, any gases evolved being allowed to escape. The membranes which are separated from the egg shells are removed by decantation. Repeated washing with water is followed by decantations between washings which result in effective removal of substantially all of the membranes from the egg shells and in removing all of the shell particles from the membranes.

The egg-shell membranes, after removal from the egg shells, are desirably washed in water or in aqueous media. For convenience in storage or subsequent handling, it is desirable to subject them to a partial or complete drying operation depending on the physical form of the product desired from the subsequent homogenization or disintegration step. If the membranes are to be disintegrated, homogenized or pulped, for example, in an aqueous medium for the production chiefly of fibrous egg-shell membrane particles, a partial removal of the water contained in the washed membranes may be accomplished simply by pressing the drained membranes between absorbent surfaces, for instance bibulous paper such as blotting paper. In this form the "blotter dry" egg-shell membranes contain about 30% by weight of membrane material, the balance being water, and they may be stored under refrigerated or freezing conditions for subsequent use in accordance with the invention. Alternatively, the drained membranes may be dried in air or in a vacuum drier and such dried membranes may be stored for prolonged periods, indeed almost indefinitely, at room temperatures without deterioration. They may be used in this form in the subsequent disintegration or homogenization step. However, in the case of dry membranes used in the disintegration or homogenization step in liquid media which are imbibed by the dry membrane and thereby soften the membrane, it is essential for best

results to allow the dry membrane material to remain with the liquid medium a sufficient time to become saturated with the liquid and softened before commencing the mechanical disintegration, homogenization or pulping action of the homogenizer or other suitable equipment. For disintegration or homogenization in liquid media which are not appreciably imbibed by the membrane and/or do not soften the membrane, the disintegrating or homogenizing action of the disintegrator or homogenizer may be started as soon as the dried membrane pieces and the suspending essentially unabsorbable liquid medium have been mixed together in the homogenizer.

The following examples are illustrative of procedures which are useful in the production of egg-shell membrane products pursuant to the invention.

EXAMPLE 1.

Preparation of Particulate Low-Bulk Product.

To 1.25 grams of air-dried, very coarsely shredded or sliced pieces of uncooked egg-shell membrane placed in the receptacle of an appropriate homogenizer approximately 170 ml of anhydrous methyl-ethyl ketone (2-butanone) are added. There is no apparent softening of the crisp membrane pieces, indicating little if any imbibition of the liquid into the structural elements of the membrane. The machine is operated at gradually increasing speeds and finally for 12 minutes at 45000 rpm while the receptacle is cooled with an ice-water bath. The pieces of membrane rapidly disintegrate and in 12 minutes the agitated liquid looks like milk. It settles rapidly, however, giving a sediment of white powder and a hazy supernatant of methyl-ethyl ketone. The sediment product appears to be composed of about equal parts of pieces of membrane and pieces of fibers. After filtration, washing out of methyl-ethyl ketone with dry acetone, or merely allowing the residual methyl-ethyl ketone to evaporate spontaneously from the collected disintegrated membrane material, an excellent particulate membrane material is obtained of great surface to weight ratio for the topical treatment of wounds. Bulk density of the disaggregated material is 0.14 grams per cc.

EXAMPLE 2.

Preparation of Felted Fibrous Sheet.

5 grams of "blotter dry" mechanically separated egg-shell membranes (equal to about 1.5 grams air-dried membranes), which prior to "blotter drying" had previously been washed, with water, are cut up into thin strips about $\frac{1}{16}$ inch to $\frac{1}{4}$ inch wide and $\frac{1}{4}$ to $\frac{3}{4}$ inch long and placed in the receptacle of an appropriate homogenizer, together with approximately 170 ml distilled water. The homogenizer is operated preliminarily (about 1 to 3 minutes) at low speed to break up the membranes and then is operated at full speed (45,000 rpm) for 13 minutes. The resulting fiber suspension is poured into a stainless steel screen box having a bottom made from stainless steel woven wire screen having 200 openings per lineal inch. The box is held perfectly level and the water is allowed to drain through the wire screen bottom; the fibres intermesh on the wire screen to form a felted mass of egg-shell membrane fiber which is then sucked down with suction and an aluminum top plate is pressed down on the sheet to assist in removing the water from on the sheet. After removing the top plate and then stopping the suction, 10 ml of a 10% w/v water solution of glycerol is carefully poured over the felted membrane fiber sheet just formed, suction is then momentarily reapplied while the top plate is pressed down on the sheet to force out as much liquid as possible. Suction is now interrupted and, while keeping the top plate in place, the screen box is placed in an inverted position on top of a conical receptacle so that the weight of the screen box and contents is supported by the pressure of the rim of the top of the receptacle against the top plate. The resulting set-up is allowed to stand overnight to allow evaporation of water through the screen interstices and the top plate is then removed leaving a dry sheet of felted egg-shell membrane fibers resting upon it and easily removable from it, having an area of 16 square inches.

The glycerol, which is desirably utilized, serves as a humectant so as to inhibit undue embrittlement or drying out of the felted sheet of egg-shell membrane fibers. In place of glycerol other humectants, generally aliphatic polyols, can be used, typical of which are sorbitol and propylene glycol. The humectant can be incorporated at any suitable stage of the process.

In the above example the egg-shell membrane fiber suspension in water may be mixed with an aqueous suspension of finely divided fibers of cotton, of cellulose, of oxy-cellulose, of alpha cellulose, of regenerated cellulose, of silk, of rayon, of wool, of nylon and of other synthetic and natural fibers. The resulting sheet will be composite as to fiber types though each side of the sheet will be similar to the opposite side. Sheets with dissimilar sides may be prepared by developing on the screen box, in the manner described, first a felted sheet of one type of fiber and subsequently developing on top of the first sheet laid down, and while the latter is still in the screen box, a second felted sheet from a different fibrous material suspension. One of the felted sheets containing egg-shell membrane fibers may, if desired, be prepared from a suspension of egg-shell membrane fibers admixed with other fibers by employing a suspension containing the mixture of fibers in the manner previously described.

EXAMPLE 3.

Preparation of Fibrous Low-Bulk Product.

1.5 grams of air-dried, mechanically separated, egg-shell membrane (equal to 5 grams "blotter dry" egg-shell membrane) together with approximately 170 ml of distilled water are placed in the receptacle of an appropriate homogenizer. After allowing the egg-shell membrane material to imbibe water so as to be in approximately equilibrium conditions with the water, the homogenizer is first operated at slow speed, as in Example 2, and then it is run at full speed for 13 minutes to convert the membrane into a fiber pulp. The resulting aqueous fiber pulp suspension is divided into two approximately equal portions each in a centrifuge bottle. The bottles so prepared are well shaken, then spun down for a period of about 10 minutes and the supernatant liquid is decanted. The residues are united in one bottle by washing one residue into the other bottle with 70 to 100 ml of acetone, the latter is then shaken to suspend the residue, then spun down for about 10 minutes in the centrifuge and the liquid again decanted. The residue is then suspended in about 100 ml acetone and collected on a Buchner suction funnel without using a filter paper. The acetone is allowed to drain away by gravity and the final residual acetone is allowed to evaporate spontaneously while preventing access of dust particles by wrapping the suction funnel in a cloth. The acetone-free, dry egg-shell membrane fiber aggregate is broken up by hand into small pieces, placed in the appropriate homogenizer in the receptacle without any liquid, and the homogenizer, after a preliminary operation of a few minutes at low speeds to break up the portions of egg-shell membrane fiber aggregate, is run at full speed for just a few seconds whereupon the egg-shell membrane fiber aggregate is completely disaggregated and the resulting separated egg-shell membrane fibers are dispersed to produce a white, extremely light fluffy product, the bulk density of which is approximately 0.0195 grams per cc. In general, utilizing the foregoing type of procedure, the bulk density of the product will usually fall within the range of 0.01 to 0.08 grams per cc. Care must be exercized in this air-fluffing operation since, if the homogenizer is run for too long a period of time, the friction set up between the egg-shell membrane and parts of the homogenizer may tend to cause some of the product to become scorched, giving an odor to the product. In general, treatment at high speed for approximately five to fifteen seconds is usually sufficient to achieve the desired results. Instead of carrying out the disaggregation or dispersion step in air, it can be done in any desired gaseous medium as, for instance, gaseous nitrogen, helium, argon, carbon dioxide and the like.

In place of acetone, other water-miscible organic solvents having specific gravities preferably less than water can be employed, typical examples of which are methyl alcohol, ethyl alcohol and methyl-ethyl ketone. Various of such materials, in addition to functioning as dehydrating media and expediting the removal of moisture, also have a sterilizing effect on the egg-shell membrane fibers. Further, suspensions of egg-shell membrane fibrous material in such water-organic solvent media may be filtered instead of centrifuged and this is a convenient way to handle larger quantities of product. Instead of removing the water or other suspending liquid from the egg-shell membrane fiber pulp suspension, in the manner described, the suspension of egg-shell membrane fibers resulting from the action of the homogenizer on the suspension of egg-shell membrane pieces, the water or other suspending liquid may be conveniently removed by subjecting the suspension of egg-shell membrane fibers to commercial drying, particularly spray-drying procedures, such as are used for the recovery of heat-labile solids from aqueous and other suspensions or solutions. The dry egg-shell membrane fiber aggregates resulting from the spray-drying operation may then be converted to the extremely light fluffy product by subjecting these aggregates to the previously described disaggregative procedure for the preparation of the extremely light fluffy product. Further, the pulverulent form of egg-shell membrane product, which may be produced in a suspension in a liquid as described, may also be recovered by such drying procedures and the aggregated pulverulent particles so produced may be disaggregated by the previously described disaggregative step.

The following table shows the results of several experiments on egg-shell membrane disintegration utilizing various organic suspending liquids in the homogenizer. The coarsely chopped membranes were allowed to stand for a time in the suspending liquid before disintegration for periods up to several minutes to permit any imbibition of liquid by the membrane to take place; 0.75 g. of the air dried membrane in 80 ml. of liquid was then subjected to the action of a homogenizer operating at a speed of 45000 rpm. The temperature was controlled by a water bath or by an ice bath around the homogenizer receptacle and the contents of the receptacle were thus kept from temperatures above 50° C. The diameters of the fibers were measured in the moist condition.

	Suspending Liquid	Time of Disintegration	Product Formed	
			Description	Size (microns)
1.	Formamide readily imbibed by membrane	5 min.	Mainly separated fibers; a few agglomerated or aggregated masses of separated fibers. No particles of membrane.	Fiber length: longest 170, shortest 24, average 67. Fiber diameter 3 to 7.
2.	Melted anhydrous Phenol reaction carried out at 45° C. (m.p. phenol=41—42 °C.). Imbibed.	3 min.	Almost entirely fibers, mostly free individual fibers but some matted agglomerated or aggregated fibers. No. pieces of original membrane.	Fiber length: longest 218, shortest 37, average 60. Fiber diameter 3 to 7.
3.	Anhydrous formic acid readily imbibed	3 min.	All fibers, no bits of original membrane. Mostly separated individual fibers but some agglomerated or aggregated mats.	Fiber length: longest 290, shortest 30, average 62. Fiber diameter 3 to 7.
4.	Glacial acetic acid readily imbibed	2.5 min.	Entirely separated fibers with a few mats of agglomerated separated fibers.	Fiber length: longest 387, shortest 43, average 100. Fiber diameter 3 to 7.
5.	Mercapto-acetic acid 98% imbibed	3.5 min.	Almost completely separated fibers; a few agglomerated mats of separated fibers.	Fiber length: longest 265, shortest 36, average 84. Fiber diameter 3 to 7.
6.	Mercapto acetic acid 80% in water imbibed	3.5 min.	Almost entirely separated fibers; a very few small agglomerations of these.	Fiber length: longest 290, shortest 36, average 90. Fiber diameter 3 to 7.
7.	Beta-mercapto propionic acid 99% Softened slowly, fair imbibition	3.5 min.	Preponderantly separated fibers. Remainder agglomerated separated fibers, small particles and occasional small bits of membrane	Mats and pieces size: 242 × 121, smallest 90 × 50. Fiber length: longest 195, shortest 22, average 98. Fiber diameter 3 to 7.
8.	Anhydrous pyridine not imbibed	3.5 min.	Product mainly small particles; a few fiber pieces, frequently stuck together. Some bits or pieces of membrane.	Size of pieces: large 25 × 19, small 16 × 13. Fiber pieces 6 × 16 when moist.
9.	2:4-Pentanedione not imbibed.	3.5 min.	Essentially a powder. A few broken bits of fibers.	Powder particles size: large 47 × 53, small 19 × 38 Fiber pieces 6 × 16 when moist.

	Suspending Liquid	Time of Disintegration	Product Formed	
			Description	Size (microns)
10.	Chloroform U.S.P. not imbibed.	6 min.	Product essentially a powder with a few fibres	Small particles 12 × 12 or less and numerous. Average particle 14 × 14. Largest bit 120 × 84. Fibers, longest 132, others 12 to 36 long. Fiber diameter 7.

The smaller average particle size obtained in experiment 10 in comparison with experiments 8 and 9 is probably related to the higher density of chloroform.

The egg-shell membrane products obtained from experiments 1 to 7 in the foregoing table can be effectively utilized in the preparation of the fluffy fiber pulp form and they may also be used effectively for the preparation of the sheet forms in the procedures as described above. Thus, for instance, the egg-shell membrane products shown in experiments 1 to 7 can be filtered off, and then (a) suspended in water and sheeted in the manner described herein, or (b) washed with acetone, air-dried and then disaggregated or fluffed to produce a fluffy fiber pulp form of egg-shell membrane product having low bulk density.

The products obtained from experiments 8, 9 and 10 in the foregoing table are suitable for use in the preparation of the powder form of egg-shell membrane product in the manner described above.

The products obtained from experiments 8, 9 and 10 may each be filtered off, washed with acetone to remove suspending liquid, air-dried and disaggregated and air-dispersed in the homogenizer to give the powder form of egg-shell membrane product according to the procedure described herein.

The manner in which the dried egg-shell membrane particulate materials of the invention are used to assist the healing of denuded areas on the human body is dependent, in part, on the nature of such areas. Thus, for example, if in the area the depth to which the tissue has been lost is relatively superficial, a sheet of felted egg-shell membrane fibers or a sheet of other felted fibers admixed with fibrous or non-fibrous particulate egg-shell membrane products described above is simply placed over said area to cover same. To this end the sheet may be cut to conform in contour to that of the area to be covered. A protective cover of hospital gauze or the like may be laid gently and preferably relatively loosely over the sheet to protect the treated lesion and to prevent contamination.

If the denuded area is quite deep, as in the case of the surgical removal of an ulcer such as a necrotic ulcer, then the area is desirably packed with the dried egg-shell membrane material. To this end, the 100% egg-shell membrane material, if in sheet form, can be cut into small pieces and the latter packed or lightly tamped into the area throughout its depth. However, in this type of situation it is particularly desirable to pack the area with the dried egg-shell membrane material as produced in disaggregated particulate powder form or in disaggregated particulate fibrous form. The area is again protected from contamination by hospital gauze as described above. As healing proceeds, additional amounts of the dried egg-shell membrane materials may be applied to the denuded area as may be indicated by the rate and degree of healing.

The egg-shell membrane felted fiber fabrics or sheets and the egg-shell membrane particulate products, both the fluffy fiber pulp form and the powder form, produced in accordance with the present invention, function, when utilized in the manner described above, as protective barriers for the denuded areas, exercising a mechanical protective action as well as markedly assisting in the healing of the lesion. The body fluids present in the tissues of the lesion may become intimately admixed with the particles of the egg-shell membrane products and on drying form a reinforced crust having considerable mechanical protective strength. Further, the enzymic substances present in such body fluids and tissue exudates may act on the egg-shell membrane particles, presenting as they do an extensive surface area for enzyme adsorption, and such enzymic action may result in the formation of substances which facilitate the healing process and/or stimulate the growth of repair tissues and ultimately of new skin covering. The dried egg-shell membrane products produced pursuant to the invention are characterized by exceptionally great surface area per unit of weight. This fact appears to play some role in relation to the manner in which said materials of the invention function in assisting the healing processes here involved, with due regard for the fact that the exact mechanism of the actions concerned in such processes is presently unknown.

It should be understood that, while it is preferred to utilize uncooked egg-shell membranes in the production of the novel egg-shell membrane products of the invention, cooked or partially cooked egg-shell membranes can be treated in the same manners as described above in connection with the uncooked egg-shell membranes to produce the particulate egg-shell membrane products as well as to produce felts or felted fiber sheets.

It has also been found that the egg-shell membrane fiber pulp prior to drying, or aqueous suspensions of the egg-shell membrane fibers or aqueous suspensions of the egg-shell membrane powder particles, prepared as described above, may be treated in aqueous media with enzymes, preferably purified, of proteolytic character, such as papain, ficin, pepsin, trypsin and trypsin related enzymes, bromelin and other proteolytic enzymes of vegetable and/or of animal origin so as to bring about partial digestion of the egg-shell membrane material. Moreover, the finished products may contain active proteolytic enzymes which serve, in certain instances, to enhance the utility of the egg-shell membrane digest product.

Useful products can also be prepared from the egg-shell membrane particulate products herein described by oxidation and also by reduction techniques whereby to modify the disulfide linkages present in the egg-shell membrane substance. Oxidations have been carried out with hydrogen peroxide, urea peroxide, peracetic acid and performic acid to oxidize disulfide groups, present in egg-shell membrane materials, to intermediate states of oxidation of the disulfide link-ages and to form cysteic acid groups. Such latter acidic groups can be reacted chemically for instance with basic amino acids such as lysine, arginine, histidine and with methionine as well as with basic peptides. Reductions have been carried out on the egg-shell membrane particulate products described herein with mercapto-acetic acid, mercapto-propionic acid, substances which contain a reactive thiol group, and their soluble salts, to convert the disulfide groups present in the egg-shell membrane products to thiol groups. The resulting reduced egg-shell membrane products can then very readily be subjected to partial digestion with proteolytic enzymes to produce modified egg-shell membrane products useful for the treatment of denuded areas on the human body.

The dried egg-shell membrane products, whether in sheet forms or in disaggregated particulate forms, can be sterilized, preferably prior to final packaging. This may be accomplished by techniques known in the art as, for example, by treatment with gaseous ethylene oxide or propylene oxide, or mixtures of each of these gases with other gases such as mixtures of ethylene oxide gas with carbon dioxide gas, preferably under pressure and at slightly elevated temperatures, for example at 40° C. to 75° C., and at pressures of 2 to 6 atmospheres for a period of 2 to 4 hours. Sterilization may also be carried out using beta-propiolactone or, in certain cases, with moist or dry heat. However, depending upon the exact manner in which the egg-sheet membrane products have been prepared and handled, they may have a practical degree of sterility without the necessity for any special sterilizing treatments.

Further, it may be advantageous under certain circumstances to combine the dried egg-shell membrane products, whether in sheet forms or in disaggregated particulate forms, with drugs, with enzymes, with antibiotics and/or with bacteriostatic agents. To this end, said dried materials may be impregnated with the desired enzyme, drug, antibiotic or bacteriostatic substance or mixtures of these either by mechanical admixing of the egg-shell membrane products with the drug in dry form or the drug may be dissolved in a suitable solvent and the drug solution applied to the egg-shell membrane products at convenient points in the preparation of the egg-shell membrane products herein described or to the finished products themselves. Typical of such antibiotics are neomycin and bacitracin, and particularly, broad spectrum antibiotics such as chlortetracycline, tetracycline, oxytetracycline, chloramphenicol, the "sulfa" drugs and others.

While the invention is especially applicable to the treatment of egg-shell membranes obtained from domestic fowl's eggs, it is also useful in relation to egg-shell membranes obtained from eggs of other birds and fowls as, for instance, duck eggs. The examples listed above were carried out with egg-shell membranes prepared from the eggs of domestic fowls because these represent the most available sources of large supplies of egg-shell membranes.

While certain preferred methods of treating egg-shell membranes have been described in detail, it will be understood that other methods of preparing products according to the invention may be apparent to those skilled in the art.

WHAT I CLAIM IS:—

1. A method of preparing an article of manufacture useful for assisting in the healing of skin-denuded areas on the human body, which comprises comminuting egg-shell membrane to produce a product having a greater total surface area than that of the same weight of egg-shell membrane.

2. A method according to claim 1, which comprises mechanically comminuting the membrane in air or in a liquid which does not soften the membrane, so as to convert it to an essentially non-fibrous product.

3. A method according to claim 2, in which the membrane is comminuted by the

action of rapidly moving cutting blades whilst it is suspended in a liquid selected from methyl-ethyl ketone, 1:4-dioxane, benzthiazol, pyridine and chloroform.

4. A method according to claim 1, which comprises mechanically comminuting the membrane in a liquid which is imbibed by and softens the membrane, so as to convert it to an essentially fibrous product.

5. A method according to claim 4, in which the membrane is comminuted by the action of rapidly moving cutting blades whilst it is suspended in a liquid selected from water, methanol, formic acid, acetic acid, propionic acid, lactic acid, phenol, chlorophenol and formamide.

6. A method according to any of claims 2 to 5, which comprises separating the comminuted product from the suspending liquid, drying the product to form aggregates, and mechanically dispersing the aggregates in a gaseous medium to form a dry fibrous or non-fibrous product of low bulk density.

7. A method according to claim 4 or 5, which comprises separating the comminuted fibrous product from the suspending liquid in the form of a felted fibrous sheet, and drying the sheet.

8. A method according to claim 7, in which the suspended fibrous product is admixed with the non-fibrous product produced according to claim 2 or 3 and the mixture is formed into the felted sheet.

9. A method according to claim 7, in which the suspended fibrous product is admixed with natural or synthetic textile fibers and the mixture is formed into the felted sheet.

10. A method according to any of claims 1 to 6 in which the comminuted fibrous or non-fibrous product or a mixture thereof is applied to one or both sides of a woven or non-woven base web to form a composite article.

11. A method according to claim 10, in which the comminuted product is attached to the fabric or plastic sheet with an adhesive.

12. A method of preparing an article of manufacture useful for assisting in the healing of skin-denuded areas on the human body, substantially as hereinbefore described with reference to the foregoing Examples.

13. An article of manufacture useful for assisting in the healing of skin-denuded areas on the human body, when prepared by a method according to any preceding claim.

14. An article of manufacture useful for assisting healing of skin-denuded areas on the human body, comprising comminuted egg-shell membrane the total surface area of which is greater than the surface area of the same weight of egg-shell membrane.

15. An article of manufacture according to claim 14, comprising comminuted membrane in an essentially non-fibrous, particulate form.

16. An article of manufacture according to claim 14, comprising comminuted membrane in an essentially fibrous form.

17. An article of manufacture according to claim 16, in which the diameter of the fibres is between 3 and 7 microns in the moist condition.

18. An article of manufacture according to claim 15, 16 or 17, in which the membrane is in a dry, disaggregated form having a bulk density between 0.01 and 0.08 g./cc.

19. An article of manufacture according to claim 16, 17 or 18, which comprises a dried, felted, fibrous sheet laid down from a suspension of the comminuted fibers.

20. An article of manufacture according to claim 19, which includes glycerol as a humectant.

21. An article of manufacture according to claim 19 or 20, which includes natural or synthetic textile fiber mixed with the comminuted fibers.

22. An article of manufacture useful for assisting in the healing of skin-denuded areas on the human body, which comprises a base web of woven or non-woven fabric, synthetic plastics material, or paper, bearing upon at least one side a felted fibrous sheet as claimed in claim 19, 20 or 21.

23. An article of manufacture useful for assisting the healing of skin-denuded areas on the human body, substantially as hereinbefore described.

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